Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 91-101, 103, 107, and 110-120 are pending in the application, with 91 and 103 being the independent claims. Claims 11-22, 63-90, 102, 104-106, and 108-109 have been cancelled without prejudice to or disclaimer of the subject matter therein. Claims 91, 103, 107, 110, 111, 116, and 117 have been amended. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Information Disclosure Statement

The Office Action stated that the English translation of a Japanese patent submitted in the IDS filed April 10, 2003 was not considered because it does not contain a date and place of publication. The Office Action also stated that,

> the date of any resubmission of any item of information contained in this information disclosure statement or the submission of any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing of the statement.

(Paper 23, at p. 2.)

Applicants respectfully point out that 37 CFR 1.98(a)(3)(ii) specifically requires that Applicants submit to the PTO any written English translation of a non-English document that is in Applicants' possession or control. Applicants could find no requirement in the rules or the MPEP that the translation have a date or place of publication in order to be considered by the PTO. Applicants consulted with Cindy Nessler, OPLA Legal Advisor, to verify that there is no requirement that the English translation have a date and place of publication in order to be considered by the Examiner. Therefore, the Office Action is in error and Applicants respectfully request consideration of the English translation, IDS Document AR37, and making the same of record in this application.

Rejections under 35 U.S.C. § 112 - Written Description

Claims 11-22 and 63-120 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. (Paper No. 23, at p. 5.) Applicants respectfully disagree.

The Court of Appeals for the Federal Circuit ("Federal Circuit") has provided substantial guidance regarding the written description requirement of 35 U.S.C. § 112 and satisfaction of the "possession test." Measured according to the Federal Circuit's extensive instructions, Applicants' specification fully supports the currently pending claims. Applicants' specification allows those of skill in the art to recognize that Applicants invented what is claimed.

The Federal Circuit has instructed that compliance with the written description requirement is to be assessed from the viewpoint of one of ordinary skill in the art. *See Vas-Cath Inc. v. Mahurkar,* 935 F.2d 1555, 1563 (Fed. Cir. 1991) ("the applicant must ... convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention"). The Federal Circuit also has instructed that compliance with the written description requirement does not require a patent specification to describe exactly the claimed subject matter; rather, the specification must show the skilled artisan that the applicant invented what is claimed. *See Union Oil Co. of Cal. v. Atlantic Richfield Co.*, 208 F.3d 989, 997 (Fed. Cir. 2000) ("The written description requirement does not require the applicant 'to describe exactly the subject matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed'"(citations omitted)). The Federal Circuit also has instructed that "[t]he disclosure rule does not require a particular form of disclosure." *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1321 (Fed. Cir. 2003).

Relative to biotechnology inventions, the Federal Circuit instructed that *functional* descriptions of biological material can satisfy the written description requirement if a structure/function correlation is known in the art. *See Amgen Inc. v. Hoechst Marion Roussel Inc.*, 314 F.3d 1313, 1332 (Fed. Cir. 2003) ("Eli Lilly did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure" (citation omitted)). Importantly, the Federal Circuit has said, in

reference to the recitation of known biological materials, "[b]oth *Eli Lilly* and *Enzo Biochem* are inapposite to this case because the claim terms at issue here are not new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend." *Amgen Inc. v. Hoechst Marion Roussel Inc.*, at 1332.

The specific references to a deposited biological material may meet the written description requirement according to the Court of Appeals for the Federal Circuit and the PTO. As the court recently stated,

> [i]n light of the history of biological deposits for patent purposes, the goals of the patent law, and the practical difficulties of describing unique biological materials in a written description, we hold that reference in the specification to a deposit in a public depository, which makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate written description of the deposited material sufficient to comply with the written description requirement of § 112, ¶1.

Enzo Biochem, Inc. v. Gen-Probe Inc., 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002); *see also*, MPEP 2163, p. 2100-160, col. 1 and p. 2100-165, col. 2 (Rev. 1, Feb. 2002).

With regard to the rejection, the Examiner has taken the position that the claims encompass 684^{20} molecules, and the specification does not disclose a representative number of species. (Paper 23, at pp. 3-4.) The Examiner also states:

> Applicants were not is [sic] possession of an M-MLV RT enzyme as a starting material for further mutational studies, and point mutations introduced into the RNAse H domain of a 684 amino acid reverse transcriptase encoded by the pRT601 vector (further referred to as

> pRT601 RT) were not proven to possess reduced RNAse H activity. It is also not clear what was the starting material for further mutational analysis.

(Paper 23, at p. 6.) In addition, the Examiner states:

> [C]laims 63, 71, 91, and 107 encompass a genus of all possible M-MLV RTs . . . and no specific amino acid sequences of any such protein or nucleic acids encoding them, including the starting material, has been presented in the specification. Thus, the definition of an M-MLV reverse transcriptase lacks any specific structure, with the protein defined solely by its function . . . . Therefore the claims fail to meet the written description requirement by encompassing sequences which are not described in the specification.

(Paper 23, at p. 7.) Applicants respectfully traverse.

The interpretation given during examination must be of a breadth consistent with the interpretation given by an artisan of ordinary skill. *In re Cortright*, 165 F.3d 1353, 1359 (Fed. Cir. 1999); MPEP 2111 at p. 2100-47 (8th ed., Rev. 1, Feb. 2003). Applicants submit that interpreting the claims to encompass 684^{20} molecules is not a reasonable interpretation because such an interpretation would include every protein 684 amino acids in length.[1] In other words, as interpreted in the Office Action, the claims encompass proteins that are totally unrelated to any reverse transcriptase much less M-

---

1 Applicants also note that the claims encompass M-MLV reverse transcriptases longer or shorter that 684 amino acids, contrary to the apparent position of the Examiner.

MLV reverse transcriptase. One of ordinary skill in the art would not interpret the claims so broadly. Therefore, the interpretation given in the Office Action is unreasonably broad.

Given a *reasonable* interpretation of the claimed genus, Applicants submit that the number of species in the specification is representative of the genus. Rather than reiterate Applicants' previous arguments along these lines, Applicants refer the Examiner to the response filed on April 3, 2003. Further, "[d]escription of a representative number of species does *not* require the description to be of such specificity that it would provide individual support for each species that the genus embraces." (emphasis added) MPEP 2163 (II)(A)(3)(a)(ii) at p. 2100-169, col. 1. Thus, contrary to the position on page 7, lines 12-13 of the Office Action, the claims may embrace sequences that are not described in the specification and still meet the written description requirements.

Concerning the alleged lack of disclosure of any M-MLV reverse transcriptase such as the starting material for mutagenesis, pRT601 was deposited at the American Type Culture Collection (ATCC) prior to the filing date of the present application and its priority application. US patent 5,017,492, at column 18, lines 41-47, discloses that pRT601 was deposited at the ATCC and was assigned ATCC accession number 67007. US patent 5,017,492 issued on May 21, 1991. Thus, pRT601 was known and publicly available at the time the present application and its priority application were filed. Applicants therefore had possession of the "starting material" for the present invention, contrary to the Examiner's position.

The Examiner also focuses on the fact the reverse transcriptase encoded by pRT601 "wasn't even an M-MLV RT enzyme, but a synthetic construct." (Paper 23, at

p. 6.) Applicants note that mutant forms of M-MLV reverse transcriptase are still considered to be M-MLV reverse transcriptases. For example, StrataScript™ (Stratagene) is a mutant form of MMLV RT that is referred to as "a novel *MMLV reverse transcriptase* that possesses no detectable RNase activity." Stratagies 16.4, p. 128 (emphasis added).[2] Thus, Applicants' characterization of the reverse transcriptase encoded by pRT601 as an M-MLV reverse transcriptase is consistent with the art.

Concerning the Examiner's assertion that "the definition of an M-MLV reverse transcriptase lacks any specific structure, with the protein defined solely by its function," paper 23, at page 7, Applicants note that a great deal is known about the structure of reverse transcriptases in general, and M-MLV reverse transcriptase in particular. Further, there is a known structure / function correlation for M-MLV reverse transcriptase. For example, Johnson *et al.* published an alignment of M-MLV, RSV, HIV and other reverse transcriptase sequences in 1986, which showed that reverse transcriptases have significant conservation between a 150-residue segment in their carboxyl termini (the RNase H domain) and a 250 residue segment in their amino termini (the polymerase domain). Johnson, M.S. *et al. PNAS USA 83*:7648-7652 (1986), p. 7649-50, figures 2-4.[3] Johnson *et al.* pointed out residues that are conserved between the sequences and identified consensus sequences and motifs. *Id.* One of the motifs Johnson *et al.* identified was the polymerase consensus motif corresponding to positions 337 to 353 of M-MLV reverse transcriptase. The authors also identified positions, for

---

2 Copy attached herewith for the convenience of the Examiner.

3 Copy attached herewith for the convenience of the Examiner.

example, those corresponding to residues 210, 216, 218, 224, 231, 235, 237, 240, 271, 274, 277, 290, 291, 309-312, and 316-317, as being identical across the aligned sequences.

Additionally, the tertiary structure of reverse transcriptases was known. For example reverse transcriptases contain a hand domain comprised of fingers, palm, thumb and connection subdomains. See, e.g., Kohlstaedt, L.A. *et al., Science 256*:1783-1790 (1992) (IDS Document AR36) and Georgiadis, M.M., *et al., Structure 3*:879-892 (1995) (IDS Document AS14). The specification describes this domain at page 19, paragraph [0058]. Moreover, Georgiadis *et al.* determined the crystal structure of a proteolytic fragment of M-MLV reverse transcriptase, and compared it to the published crystal structure of HIV reverse transcriptase. They found that the overall fold and structures of the fingers and palm domains in M-MLV and HIV-1 reverse transcriptase are very similar. Georgiadis *et al.*, p. 883, col. 1.

Georgiadis *et al.* found that the fingers domain of M-MLV reverse transcriptase is composed of a highly twisted five-stranded mixed sheet, three α helices, and two β hairpin structures, one of which is part of the sheet. Georgiadis *et al.*, p. 880, col. 2. The palm domain of M-MLV reverse transcriptase is composed of a four-stranded antiparallel β sheet and two long α helices in the core of the domain, a β hairpin in the primer grip region, two short α helices, a short 3_{10} helix, and another short α helix. *Id.*, p. 881, col. 1. The fingers and palm domains also contain conserved resides including Lys53, Gln63, Ser195, and Gln260 of M-MLV reverse transcriptase. *Id.*, p. 881, col. 2.

At the junction of the fingers and palm domains lies the polymerase active site. *Id.*, p. 884, col. 1. This site contains three Asp residues (at positions 150, 224 and 225 of

M-MLV reverse transcriptase) that are required for polymerase activity. *Id.* The polymerase site in M-MLV reverse transcriptase contains a type II' turn. *Id.*, p. 885, col.2. HIV-1 reverse transcriptase also contains a type II' turn at the equivalent site. *Id.* In other proteins, position 2 of type II' turns is commonly a Gly residue but in all known wild-type reverse transcriptases, there is a non-Gly residue at this position. *Id.* The residues and interactions that stabilize this structure were also identified. *Id.*, p. 885, col.2 to p. 886, col. 1.

Georgiadis *et al.* also used the crystal structure of M-MLV and the conserved residues across murine, avian and human reverse transcriptases to identify the structures involved in fidelity, processivity, and selectivity for dNTPs. For example, Georgiadis *et al.* state:

> the highly conserved residues Gln190 (151 in HIV-1 RT) and Gly191 (152 in HIV-1 RT) found in the conserved sequence LPQG within loop β_9-α_H (part of motif B, a conserved sequence found on RTs []), form hydrogen bonds in the minor groove to O2 or N3 of the dNTP and template base, respectively.

Id., p. 886. col. 2. They also state that conserved residues Lys103, Arg 110, and Asp114 interact with the template strand, and Arg116 and Asn119 interact with the primer strand. *Id.*, p. 887, col. 2. With regard to selectivity for dNTPs, Georgiadis *et al.* state that the interaction between Phe155 and the 2'-hydroxyl of a ribose nucleotide disfavors rNTP, and that Tyr - the only other residue found at that position in reverse transcriptases - would result in the same selectivity as Phe. *Id.*, p. 888, col. 1. Based on the results of Georgiadis *et al.*, Gao *et al.* mutated Phe155 and determined that a substitution with

valine allows reverse transcriptase to incorporate rNTPs. Gao, G. *et al., Proc. Natl. Acad. Sci. USA 94*:407-411 (1997) (IDS Document AR14). Clearly, as the evidence above shows, there is a known structure / function correlation for M-MLV reverse transcriptase.

Concerning the Examiner's assertion that the specification does not prove that the point mutations made in the RNAse H domain reduce RNAse H activity, Applicants respectfully point out that it is the Examiner who bears the burden of casting doubt on Applicants' presumptively accurate disclosure. The Examiner has failed to bear this burden. Moreover, Applicants provided evidence of the reduced RNAse H activity in the response filed on April 3, 2003.

While the examined claims comply fully with the written description requirement of §112, Applicants nonetheless have amended the claims in an effort to advance prosecution. As amended, the claims recite M-MLV reverse transcriptase having mutations at particular positions.

In view of the above remarks, Applicants respectfully request that this rejection be withdrawn.

Rejections under 35 U.S.C. § 112 - Enablement

Claims 11-21 and 63-120 were rejected under 35 U.S.C. §112, first paragraph, because the specification is allegedly not enabled for the full scope of the claims. (Paper 23, at p. 7). Applicants traverse this rejection.

The Examiner contends that:

> [d]ue to the large quantity of experimentation necessary to determine all possible mutations in all possible M-MLV reverse transcriptases which will result in increased enzyme fidelity, the lack of direction and guidance presented in the specification regarding creation of all possible mutations in all possible M-MLV reverse transcriptases which will result in increased enzyme fidelity, the absence of working examples directed to making such mutations in M-MLV reverse transcriptases, the unpredictability of the effects of mutations on protein structure and function (see references below), undue experimentation would be required of the skilled artisan to make and use the claimed invention in its full scope.

Office Action, page 9, lines 14-21.

In support of the enablement rejection, the Office Action cites a number of publications including Jin *et al.* and Chowdhury *et al.* Applicants submit that these references do not provide a reasonable basis to doubt the scope of enablement of the pending claims. However, the claims have been amended to recite mutations at particular residues of M-MLV reverse transcriptase. As amended, the claims do not recite mutations at position Arg110, or the mutations Val223Met, Val223Ser, Val223Ala, Val223Ile, and Tyr598Val. Therefore, Kaushik *et al.,* Chowdhury *et al.*, and Halvas *et al.* do not support the rejection of the pending claims.

In view of the above remarks, Applicants respectfully request that this rejection be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 71, 82, 91, and 102 were rejected under 35 U.S.C. §102 as allegedly being anticipated by Halvas *et al.*, *J. Virology 74*:312-319 (Jan. 2000). Applicants respectfully traverse the rejection.

Applicants have cancelled claims 71, 82, and 102 and have amended claim 91 so as not to recite a mutation at the position Val223. Therefore, Halvas *et al.* do not teach or suggest the pending claims. Accordingly, the rejection has been rendered moot. Withdrawal is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Helene C. Carlson
Agent for Applicants
Registration No. 47,473

Date: 1/2/2004

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

204136v2

Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7648–7652, October 1986
Biochemistry

Computer analysis of retroviral *pol* genes: Assignment of enzymatic functions to specific sequences and homologies with nonviral enzymes

(ribonuclease H/reverse transcriptase)

M. S. JOHNSON, M. A. MCCLURE, D.-F. FENG, J. GRAY, AND R. F. DOOLITTLE

Department of Chemistry, University of California at San Diego, La Jolla, CA 92093

Contributed by R. F. Doolittle, June 30, 1986

ABSTRACT **A computer analysis of the amino acid sequences from the putative gene products of retroviral *pol* genes has revealed a 150-residue segment that is homologous with the ribonuclease H of *Escherichia coli*. The segment occurs at the carb xyl terminus of the region assigned to the 90-kDa reverse transcriptase polypeptide. In contrast, a section nearer the amin terminus of this sequence can be aligned with nonretroviral polymerases. The order of activities in the *pol* gene is thus: polymerase–ribonuclease–endonuclease. On another note, all retroviral endonuclease sequences contain a consensus zinc-binding "finger." This should not be confused with the well-known zinc requirement of reverse transcriptases.**

We have been conducting a computer analysis of retroviral protein sequence relationships. During the course of this study, we uncovered a number of unexpected features among the inferred products of the retroviral polymerase gene. In particular, we identified sequences in the *pol* gene products that are clearly related to some nonretroviral enzymes. The results lead to a functional arrangement of activities in the *pol* gene region that differs from that reported by others (1–3). The activities that are under scrutiny here and that are encompassed by the *pol* gene include: the RNA-directed DNA polymerase (reverse transcriptase; EC 2.7.7.49) (4), a ribonuclease H that degrades the viral RNA in the immediate wake of its reverse transcription (5), and an endonuclease ("integrase") that is essential for the integration of the newly synthesized DNA into the host genome (6).

The *pol* gene of retroviruses is expressed initially as a gag–pol precursor that is proteolytically processed to a number of small gag proteins, an approximately 90-kDa protein encompassing both RNA-directed DNA polymerase (reverse transcriptase) and ribonuclease H activities, and, finally, a 40-kDa fragment with endonuclease activity (7). Several reports have presented evidence that the ribonuclease H activity of the 90-kDa reverse transcriptase portion is associated with the amino-terminal end of that protein, and by implication, that the DNA polymerase activity is at the carboxyl-terminal end. These conclusions are based on experiments involving deletion mutants (2), on the one hand, and antibodies to synthetic peptides modeled on the putative sequences, on the other (3).

We now suggest that the opposite must be true: the ribonuclease H activity should be situated at the carboxyl terminus, and the DNA polymerase, at the amino terminus. We draw this conclusion on the basis of comparisons of the retroviral sequences with those of nonviral enzymes of similar function. In this regard, we have uncovered a significant resemblance between a 150-residue segment at the carboxyl-terminal end of the 90-kDa fragment and the reported sequence of a ribonuclease H from *Escherichia coli*. We also provide an alignment of a segment near the amino terminus of the 90-kDa polypeptide with highly conserved sequences from many other polymerases, including the α subunit of *E. coli* DNA-directed RNA polymerase. Finally, there is a distinctive sequence in the endonuclease sequence that is characteristic of a zinc-binding segment.

METHODS

The sequences used were taken from the 1985 version of NEWAT (8) or release 6.0 of the National Biomedical Research Foundation Atlas (9). The particular versions of the retroviral sequences employed are: human T-cell leukemia virus type I (HTLV-I), Seiki *et al.* (10); bovine leukemia virus (BLV), Rice *et al.* (11); Rous sarcoma virus (RSV), Schwartz *et al.* (12); mouse Moloney leukemia virus (Mo-MLV), Shinnick *et al.* (13); human immunodeficiency virus (HIV; formerly HTLV-III/LAV), Ratner *et al.* (14); *E. coli* ribonuclease H, Kanaya and Crouch (15); and the α subunit of *E. coli* DNA-directed RNA polymerase, Ovchinnikov *et al.* (16).

The search program used a moving window of 40 residues and a table of weighted values taken from the mutation matrix of Dayhoff *et al.* (17). Alignments were performed with programs based upon the original algorithm of Needleman and Wunsch (18) as described by Feng *et al.* (19).

RESULTS

Ribonuclease H and Polymerase Sequences. The sequence of ribonuclease H from *E. coli* resembles the carboxyl-terminal portion of retroviral reverse transcriptases. In the case of the Mo-MLV comparison, the two segments are 30% identical (Fig. 1). Binary comparison of each of the retroviral sequences with the *E. coli* ribonuclease H sequence, followed by statistical evaluation by a randomization method, gave authentic alignment scores from 4 to 10 standard deviations above the means of the jumbled comparisons. The cumulative weight of the multiple alignment (Fig. 2) further bears out the significance of the overall relationship.

That the polymerase portion of the viral reverse transcriptase system must encompass the amino-terminal portion of the 90-kDa fragment is established by the alignment shown in Fig. 3. The key region here involves a sector previously shown by Kamer and Argos (20) to be present in a number of nonretroviral polymerases; these consistently have two aspartic acid residues surrounded by a set of nonpolar amino acids. To make the point further, we added the sequence of the α subunit of *E. coli* DNA-directed RNA polymerase to the alignment (Fig. 3).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Mo-MLV, mouse Moloney leukemia virus; HIV, human immunodeficiency virus; HTLV-I, human T-cell leukemia virus, type I; BLV, bovine leukemia virus; RSV, Rous sarcoma virus.

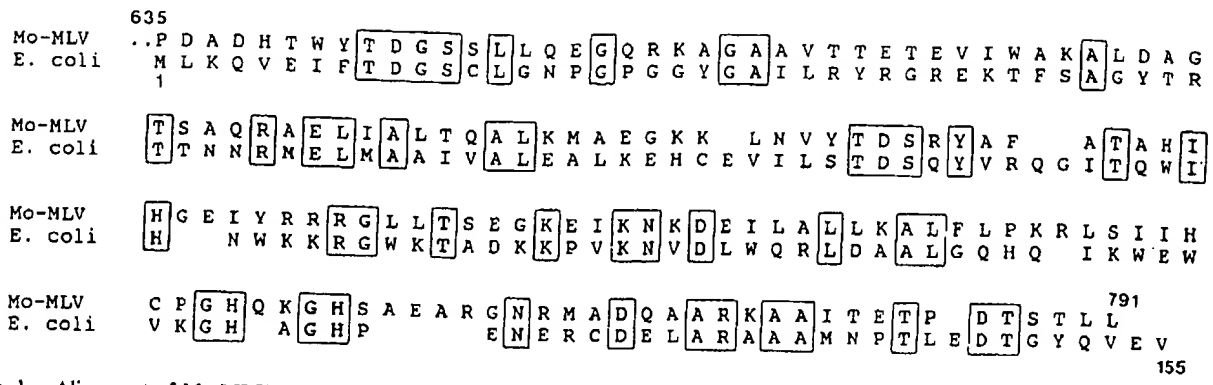

FIG. 1. Alignment of Mo-MLV and *E. coli* ribonuclease H (E. coli). Residues 635–791 of Mo-MLV correspond to the carboxyl-terminal portion of the reverse transcriptase/RNase H portion of the *pol* gene as diagrammed in Fig. 6. The aligned sequences are identical in 30% of the positions as indicated by the boxed residues.

It is known that limited proteolytic digestion of the 90-kDa fragment can give rise to smaller polypeptides with only ribonuclease H activity (21), implying that the two functions exist in two quite different settings. In this regard, the retroviral sequences of the *pol* system are highly conserved over the course of their first 250 residues, a section of about the same dimensions as the α subunit of *E. coli* polymerase, and over the course of their carboxyl-terminal 150 residues, a segment approximating the length of the *E. coli* ribonuclease H protein. Between these two conserved regions, however, is a 200-residue sector that is considerably more variable from one retrovirus to another. As such, it seems to us a good candidate for a connecting tether between the regions embodying the two enzymatic activities.

The Endonuclease Fragment. A thorough search of our data bases did not reveal any obvious relationships between retroviral endonucleases and other proteins, although an intriguing, albeit marginal, resemblance is discernible with a portion of an *E. coli* "transposase" (22). On the other hand, analysis of the retroviral endonuclease sequences did reveal the presence of a constellation of amino acids recently reported to be diagnostic for a zinc-binding site of the sort that can interact with DNA (23–25). In this case, the consensus, which is rigorously conserved in all of the retroviral endonucleases we have examined (upwards of a dozen), involves two histidines separated by 20–30 residues from a brace of closely spaced cysteines (Fig. 4). It has been postulated that the zinc is tetrahedrally coordinated by the histidyl and cysteinyl sidechains and that the residues between the two sets of ligands exist as a ribbon that can wrap around the DNA strand (23). A depiction of the endonuclease segment from HIV is presented in such a form in Fig. 5. The zinc predicted on the basis of this sequence should not be confused with the demonstrated zinc of reverse transcriptases (26, 27); the latter presumably acts in a catalytic fashion in all polymerases.

A number of other residues are highly conserved in the retroviral endonucleases, although the degree of conservation falls off markedly near the carboxyl terminus. This is taken to an extreme in the Mo-MLV sequence, in which case a 36-residue intrusion occurs; so far this segment has not been seen in any of the other retroviral sequences.

DISCUSSION

Several laboratories have reported data that have been interpreted as indicating that the ribonuclease H activity is near the amino-terminal end of the 90-kDa reverse-transcriptase fragment (1–3). As a result, some investigators

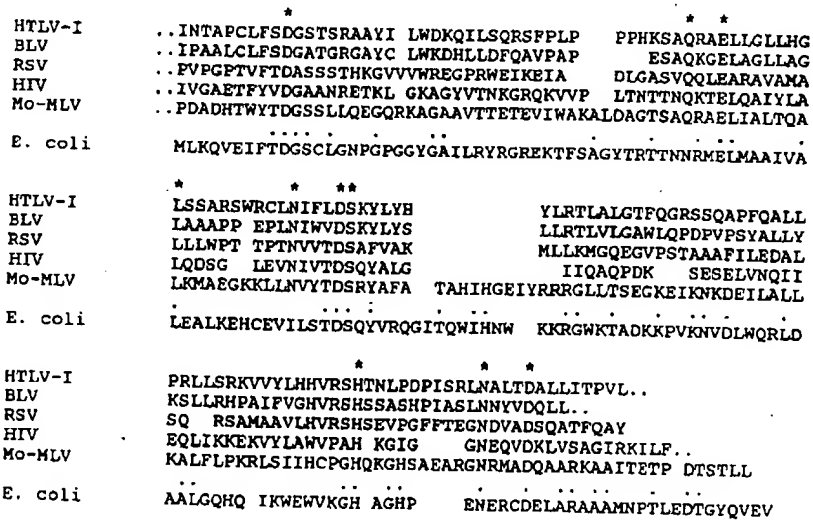

FIG. 2. Alignment of the full-length 155-residue *E. coli* ribonuclease H sequence (E. coli) with five retroviral protein sequences derived from the *pol* genes: HTLV-I, residues 465–599; BLV, 432–557; RSV, 441–572; HIV, 601–728; and Mo-MLV, 635–791. For the HIV and the Mo-MLV sequences, the long open reading frame encodes the protease, which is followed by the *pol*; residue numbering is based on the first position of this long open reading frame. These sequences correspond to the carboxyl-terminal region of the reverse transcriptase/RNase H sequences as depicted in Fig. 6; the experimentally determined carboxyl-terminal residues for RSV, Mo-MLV (7), and *E. coli* RNase H are in fact the last residues shown in the alignment. Asterisks above residues indicate identities in all five of the *pol* sequences; dots between the *E. coli* and the Mo-MLV sequence denote identical residues.

FIG. 3. A portion (residues 117–329) of the 329-residue *E. coli* α subunit of DNA-directed RNA polymerase (E. coli) is aligned with amino-terminal segments of retroviral pols. The match centers around the polymerase consensus "Asp-Asp" (19) sequence (dashed underline). The reverse transcriptase from HTLV-I (residues 33–279), BLV (7–253), RSV (27–269), HIV (194–439), and Mo-MLV (187–436) are derived from the reverse transcriptase/RNase H sequence as shown in Fig. 6. Asterisks indicate positions where each of the five retroviral sequences have identical residues; dots denote residues in common to both Mo-MLV and the *E. coli* sequence. See Fig. 2 for the identification of sequence codes.

report their results in the context of a "ribonuclease–polymerase–endonuclease" arrangement (28, 29). At the same time, other workers, perhaps unaware of these assignments, have clearly shown that segments of less than 200 residues from the amino terminus can be aligned with portions of nonretroviral polymerases, including those from hepatitis B virus and cauliflower mosaic virus (30), as well as from tobacco mosaic and brome mosaic viruses and several picornaviruses (20). As far as is known, the latter do not exhibit ribonuclease H activity.

The question arises: what could have misled some workers into thinking that the ribonuclease activity is near the amino terminus? The problem seems to have two roots. In the one case, experiments involving a murine leukemia virus mutant with a frameshift in the *pol* gene region revealed that premature chain termination gave rise to a truncated poly-

FIG. 4. Alignment of the endonuclease sequences of retroviral *pol* sequences: HTLV-I (residues 600–896), BLV (558–852), RSV (573–895), HIV (732–1015), and Mo-MLV (841–1199). The sequences contain a pair of histidines (lower-case letters) and cysteines (lower-case letters) in the amino-terminal portion of the sequences that may coordinate a zinc metal ion (dashed underline) and form a nucleotide binding finger (see Fig. 5). The amino-terminal residue of RSV shown in the alignment is known to be the amino-terminal residue of that endonuclease (7), as is depicted in Fig. 6; in each case, the putative carboxyl-terminal residues that complete the sequence are based on the stop codon that terminates each pol polyprotein. Asterisks have been placed above residues that are identical in each of the five sequences. See Fig. 2 for the identification of sequence codes.

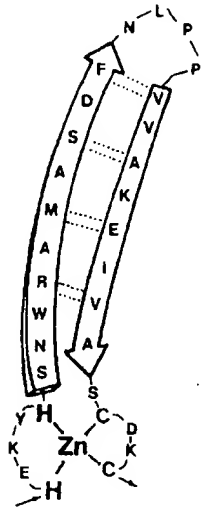

FIG. 5. Ribbon drawing of the proposed zinc metal-binding finger for the retroviral endonuclease of HIV virus that is essential for integration of newly synthesized DNA into the host genome. Dotted lines indicate potential hydrogen bonds oriented along a presumed β structure. Arrows indicate the direction of the chain from the amino to the carboxyl terminus.

merase about two-thirds of normal size. In line with their main goal, these workers accurately demonstrated that the retroviral protease activity must be upstream of the mutated region. They extended their interpretation, however, to include a presumption about the location of the ribonuclease H. They noted that a small amount of transcription took place in their system, and this led them to believe that both ribonuclease H and polymerase activities must lie within the amino-terminal portion of the polymerase protein. It should be pointed out, however, that second-strand synthesis was not reported by those authors, a situation that would be consistent with the absence of ribonuclease H activity.

Another set of experiments to the contrary involved a protein fragmentation study in conjunction with antibodies to synthetic peptides. Grandgenett *et al.* (3) synthesized a series of peptides corresponding to various parts of the *pol* gene product sequence and raised antibodies to them. They then fragmented the equivalent of the 90-kDa fragment from RSV by a route previously reported to yield ribonuclease H activity associated with a 24-kDa fragment (22). In fact, their antibodies to peptides based on the amino-terminal region reacted with a fragment that they presumed to be 24 kDa. What was not commented on, however, but is clearly shown in the published photograph (3) is that antibodies to peptides from the carboxyl-terminal region of the 90-kDa fragment reacted with a somewhat smaller component, consistent with what might be expected for the approximately 150-residue sequence we have assigned to the ribonuclease H.

It can also be asked how it was that workers who reported the *E. coli* ribonuclease H sequence did not notice the resemblance to retroviral sequences. In fact, Kanaya and Crouch (15) compared the *E. coli* sequence to that of the RSV *pol* gene product, but the computer dot-matrix method they used (31) was apparently not sensitive enough to bring out the similarity.

It should be noted that the ribonuclease H from *E. coli* is an endonuclease, whereas the ribonucleases H from retroviruses are exonucleases (1). Similarly, the *E. coli* polymerase that we have aligned with the amino-terminal region of the retrovirus *pol* gene product is a DNA-directed RNA polymerase. These differences notwithstanding, the similarities in sequence are compelling (Figs. 1 and 2), and we contend that the arrangement of activities in the retroviral *pol* gene is "polymerase–ribonuclease–endonuclease." Moreover, the polymerase and ribonuclease functions are separated by a poorly conserved region that may be a tether between two better-defined structures (Fig. 6). The basis for these functional assignments is the similarity in sequence to nonviral enzymes of similar function.

We thank Inder Verma for helpful comments on the manuscript. This work was supported by a grant from the American Cancer Society and National Institutes of Health Grant GM-34434.

1. Crouch, R. J. & Dirksen, M.-L. (1982) in *Nucleases*, eds. Linn, S. M. & Roberts, R. J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 211–241.
2. Levin, J. G., Hu, S. C., Rein, A., Messer, L. I. & Gerwin, B. I. (1984) *J. Virol.* **51**, 470–478.
3. Grandgenett, D., Quinn, T., Hippenmeyer, P. J. & Oroszlan, S. (1985) *J. Biol. Chem.* **260**, 8243–8249.
4. Temin, H. M. & Mizutani, S. (1970) *Nature (London)* **226**, 1211–1213.
5. Verma, I. M. (1975) *J. Virol.* **15**, 121–126.
6. Schwartzberg, P. J., Colicelli, J. & Goff, S. P. (1984) *Cell* **37**, 1043–1052.

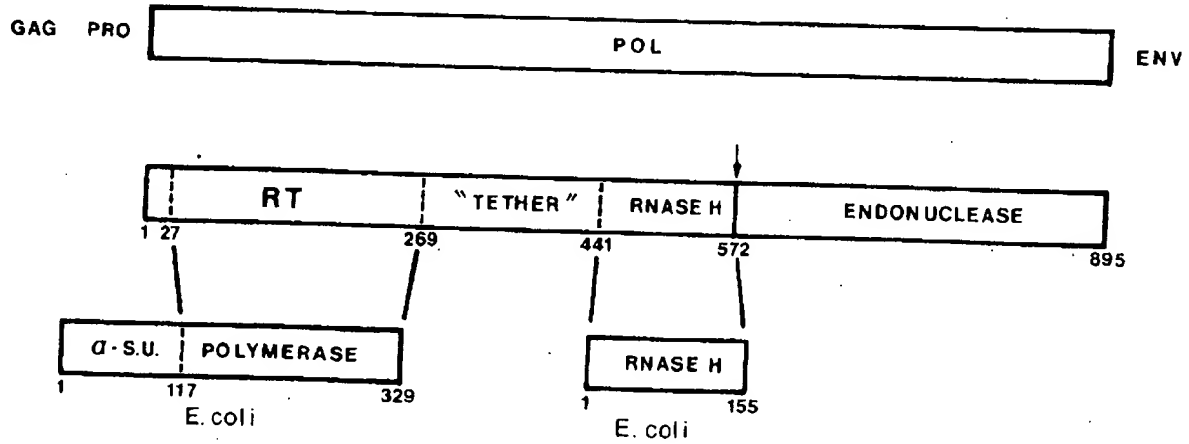

FIG. 6. Postulated map of the retroviral *pol* gene. The *pol* gene is between the *gag* (GAG)-protease (PRO) genes and the *env* (ENV) gene. The protein product, shown in this case for RSV, is depicted with functional assignments assigned to specific regions of the 895-residue product. The RSV pol polyprotein is known to be proteolytically cleaved at a Tyr–Pro bond (arrow), giving rise to the reverse transcriptase (RT)/RNase H polypeptide and the endonuclease (7). The RNase H was localized to the carboxyl terminus of the RT/RNase H protein by virtue of its homology with the RNase H of *E. coli*; similarly, the *E. coli* α subunit of RNA-directed DNA polymerase can be aligned with residues at the amino terminus of the polypeptide.

7. Van Beveren, C., Coffin, J. & Hughes, S. (1985) in *RNA Tumor Viruses/Supplements and Appendixes*, eds. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), 2nd Ed., pp. 589–594; pp. 773–779.
8. Doolittle, R. F. (1981) *Science* **214**, 149–159.
9. George, D. G., Barker, W. C. & Hunt, L. T. (1986) *Nucleic Acids Res.* **14**, 11–15.
10. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3618–3622.
11. Rice, N. R., Stephens, R. M., Burny, A. & Gilden, R. V. (1985) *Virology* **142**, 357–377.
12. Schwartz, D. E., Tizard, R. & Gilbert, W. (1983) *Cell* **32**, 853–869.
13. Shinnick, T. M., Lerner, R. A. & Sutcliff, J. G. (1981) *Nature (London)* **293**, 543–548.
14. Ratner, L., Haseltine, W., Patarca, R., Livak, K. J., Starcich, B., Josephs, S. F., Doran, E. R., Rafalski, J. A., Whitehorn, E. A., Baumeister, K., Ivanoff, L., Pettewat, S. R., Jr., Pearson, M. L., Lautenberger, J. A., Papas, T. S., Ghrayeb, J., Chang, N. T., Gallo, R. C. & Wong-Staal, F. (1985) *Nature (London)* **313**, 277–284.
15. Kanaya, S. & Crouch, R. J. (1983) *J. Biol. Chem.* **258**, 1276–1281.
16. Ovchinnikov, Yu. A., Lipkin, V. M., Modyanov, N. N., Chertov, O. Yu. & Smirnov, Yu. V. (1977) *FEBS Lett.* **76**, 108–111.
17. Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. (1978) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (Nat. Biomed. Res. Found., Washington, DC), Vol. 5, Suppl. 3, pp. 345–358.
18. Needleman, S. B. & Wunsch, C. D. (1970) *J. Mol. Biol.* **48**, 443–453.
19. Feng, D.-F., Johnson, M. S. & Doolittle, R. F. (1985) *J. Mol. Evol.* **21**, 112–125.
20. Kamer, G. & Argos, P. (1984) *Nucleic Acids Res.* **12**, 7269–7282.
21. Lai, M.-H. T. & Verma, I. M. (1978) *J. Virol.* **25**, 652–663.
22. Heffron, F., McCarthy, B. J., Ohtsubo, H. & Ohtsubo, E. (1979) *Cell* **18**, 1153–1163.
23. Miller, J., McLachlan, A. D. & Klug, A. (1985) *EMBO J.* **4**, 1609–1614.
24. Hartshorne, T. A., Blumberg, H. & Young, E. T. (1986) *Nature (London)* **320**, 283–287.
25. Berg, J. M. (1986) *Science* **232**, 485–487.
26. Poiesz, B. J., Battula, N. & Loeb, L. A. (1974) *Biochem. Biophys. Res. Commun.* **56**, 959–964.
27. Auld, D. S., Kawaguchi, H., Livingston, D. M. & Vallee, B. L. (1975) *Biochem. Biophys. Res. Commun.* **62**, 296–302.
28. Dunwiddie, C. & Faras, A. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5097–5101.
29. Freeman-Wittig, M.-J., Vinocour, M. & Lewis, R. A. (1986) *Biochemistry* **25**, 3050–3055.
30. Toh, H., Hayashida, H. & Miyata, T. (1983) *Nature (London)* **305**, 827–829.
31. Maizel, J. V., Jr., & Lenk, R. P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7665–7669.

OCTOBER 1986
VOLUME 83
NUMBER 20

Proceedings
OF THE
National Academy of Sciences
OF THE UNITED STATES OF AMERICA

Proceedings
OF THE
National Academy of Sciences
OF THE UNITED STATES OF AMERICA

Editorial correspondence: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, 2101 Constitution Avenue, Washington, DC 20418.

Business correspondence: Circulation Office of the PROCEEDINGS, National Academy of Sciences, 2101 Constitution Avenue, Washington, DC 20418.

Information for Contributors: See issue Number 1, January 1986.

Subscriptions: All correspondence concerning subscriptions should be addressed to the Circulation Office of the PROCEEDINGS. Subscriptions are entered on a calendar year basis only. For 1986, subscription rates are as follows—in the United States: student/postdoctoral, $65; personal, $185; institutional, $215; elsewhere: student/postdoctoral, $100; personal, $220; institutional, $250. Subscribers are requested to notify the Circulation Office of the PROCEEDINGS 6 weeks in advance of any change of address; also the local postmaster. The Academy is not responsible for nonreceipt of issues because of an improper address unless a change of address is on file. The notice of address change should list both the old and new addresses. Claims for replacement copies will not be honored more than 60 days after the issue date for domestic subscribers and not more than 90 days after the issue date for foreign subscribers.

Back Issues: Volumes 78–82, January 1981 and thereafter, are available from the Circulation Office of the PROCEEDINGS. The price of a single issue is $21.00 for Volume 78 or $11.00 for Volumes 79–83.

Second class postage paid at Washington, DC, and at additional mailing offices. PRINTED IN THE USA

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (ISSN-0027-8424) *is published semimonthly by* THE NATIONAL ACADEMY OF SCIENCES, *2101 Constitution Avenue, Washington, DC 20418.*

POSTMASTER: Send address changes to: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 2101 Constitution Ave., Washington, DC 20418.

- RNASE DEFICIENCY GENERATES LONGER TRANSCRIPTS
- NUCLEASE-FREE ENZYME PRODUCES ROBUST CDNA YIELDS
- SAME GREAT PERFORMANCE AS A LEADING COMPETITOR

Great All Around RT Performance

StrataScript™ reverse transcriptase delivers excellent performance for applications requiring a reverse transcriptase. Because StrataScript reverse transcriptase is genetically enhanced to eliminate RNAse H activity, it produces full-length cDNA with the greatest yield and can be used in many applications.

RNAse H activity degrades precious RNA template molecules resulting in short cDNA molecules. This makes it difficult to clone full-length genes, generate labeled target RNA for microarray analysis, and effectively produce cDNA for QPCR assays.

Generate Full-Length cDNA

StrataScript™ reverse transciptase is a novel MMLV reverse transcriptase that possesses no detectable RNAse activity.[1] This mutant features a point mutation introduced into wild-type MMLV-RT gene, effectively inactivating the RNAse H active site normally associated with wild type MMLV, but not affecting the desired RT function. This allows you to easily and efficiently produce full-length cDNA, which results in successful gene cloning experiments.

Nuclease-Free Means More cDNA

Because Stratagene manufactures StrataScript RT with the highest quality, we guarantee that endonucleases, exonucleases, and non-specific RNAse activities are absent in every batch. This ensures StrataScript RT produces more robust yields of cDNA transcripts compared to MMLV and RNase H+ reverse transcriptases. Performance is equivalent to SuperScript RT (Figure 1). As a result of stringent quality control, StrataScript RT is ideal for use in the most demanding and sensitive applications such as QRT-PCR and microarray target labeling.

Optimized Buffer Included in Every Kit

StrataScript RT enzyme is supplied with a 10X buffer specifically optimized for reverse transcription reactions, making it easy to set up your RT reactions and allowing you to get results fast—all without tedious troubleshooting of buffer conditions.

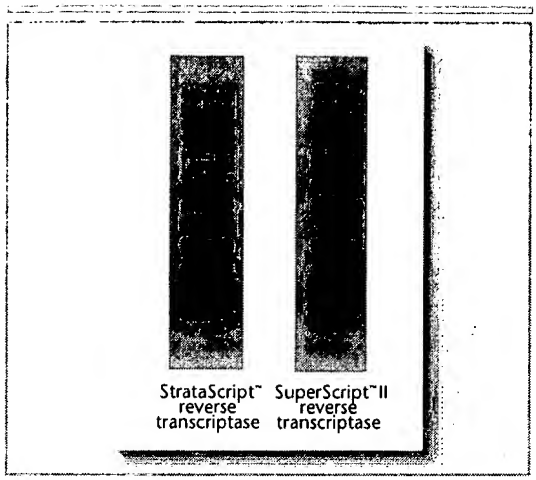

Figure 1
StrataScript™ Reverse Transcriptase Delivers Same Robust Performance as SuperScript™ II Reverse Transcriptase
Reactions using 100 U StrataScript™ reverse transcriptase and 100 U SuperScript™ II reverse transcriptase generated equivalent performance at 40°C.

Every Application Counts

Reverse transcriptases are used to generate cDNA for many applications such as RT-PCR, microarray target labeling, QRT-PCR, cDNA library construction, and 5'-RACE. StrataScript RT is featured in many of our premiere products for these applications. We include StrataScript RT in our FairPlay™ microarray labeling kit for high-efficiency microarray target labeling, Brilliant® reagent kits for robust and sensitive QRT-PCR, and StrataScript RT-PCR systems for high-fidelity RT-PCR experiments.

REFERENCE:
Nielson, K., et al. *Strategies* 6: 45-46.

15% OFF!*

* *See inside front cover for more details.*

StrataScript™ Reverse Transcriptase	Catalog
10,000 U (50 U/µl)	#600085
10,000 U (200 U/µl)	#600086 NEW
StrataScript™ One-Tube RT-PCR System	Coming Soon! NEW
StrataScript™ Two-Tube RT-PCR System	Coming Soon! NEW
For more information on our new RT-PCR systems, please visit www.stratagene.com/RT	

NEW

Protein Function and Analysis

15% OFF!*

Overcome Mutational Bias During Random Mutagenesis

GeneMorph® II Random Mutagenesis Kit

- Includes Mutazyme® II DNA polymerase specifically engineered to overcome mutational bias
- Simple protocol to control mutation frequency from 1 to 7 mutations per kb
- Robust yields of targets 1 to 6 kb for easy cloning and library construction

The GeneMorph® II random mutagenesis kit* takes error-prone PCR to the next level by combining mutational spectrums of both our Mutazyme® DNA polymerase* and *Taq* DNA polymerase. The kit contains a genetically enhanced version of Mutazyme DNA polymerase, which introduces a more uniform mutational spectrum in which mutations at As and Ts occur at the same frequency as Gs and Cs (Figure 1). This allows you to generate all types of random mutations and more rapidly discover key sites responsible in structure-function relationships. This enhanced enzyme also produces robust yields, making cloning and library construction easier than ever.

GeneMorph® II Random Mutagenesis Kit
30 reactions #200550

* See license reference 9 on page 136.

** *See inside back cover for more details.*

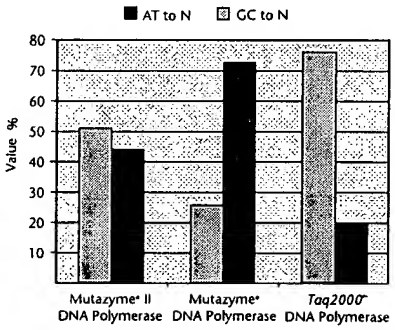

Figure 1
Mutazyme® II Polymerase Delivers Ideal Mutational Spectrum
Mutazyme® II DNA polymerase introduces a more uniform mutational spectrum in which mutations at As and Ts occur at the same frequency as Gs and Cs. This approach produces a more comprehensive collection of mutants to discover key functional sites. On the other hand, *Taq* polymerase under error-prone conditions and the original Mutazyme® DNA polymerase deliver bias mutational spectra and many key sites often would go undiscovered.